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## Introduction

### *Campylobacter jejuni*:

- ▶ Leading cause of foodborne diarrheal disease worldwide.
- ▶ Main reservoir: poultry

### Essential Oils:

- ▶ Antimicrobial properties via membrane permeability disruption.
- ▶ Often absorbed before they reach the last part of the intestinal tract.
- ▶ Carvacrol is effective against *Campylobacter jejuni*.

## Objectives

- ▶ Creation of a formulation helping the carvacrol to reach the caeca [*Campylobacter* location]. This product contains a liquid formulated core based on carvacrol, and a specific solid carrier.
- ▶ Determination of the efficacy and the mode of action of the liquid formulated carvacrol compared to the pure carvacrol.

## Experimental approach

### EFFICACY OF THE PRODUCTS:

Use of the broth microdilution method in 96-well plates to determine the minimum inhibitory concentration (MIC) and the half maximal inhibitory concentration (IC<sub>50</sub>) of gentamicin and carvacrol alone or formulated on a reference strain of *Campylobacter jejuni* (ATCC 33291)  
→ Test of 12 concentrations of the 3 products simultaneously.

### STATISTICAL ANALYSIS:

Values compared with a Kruskal-Wallis test and pairwise differences further analysed using a Mann-Whitney-Wilcoxon test.

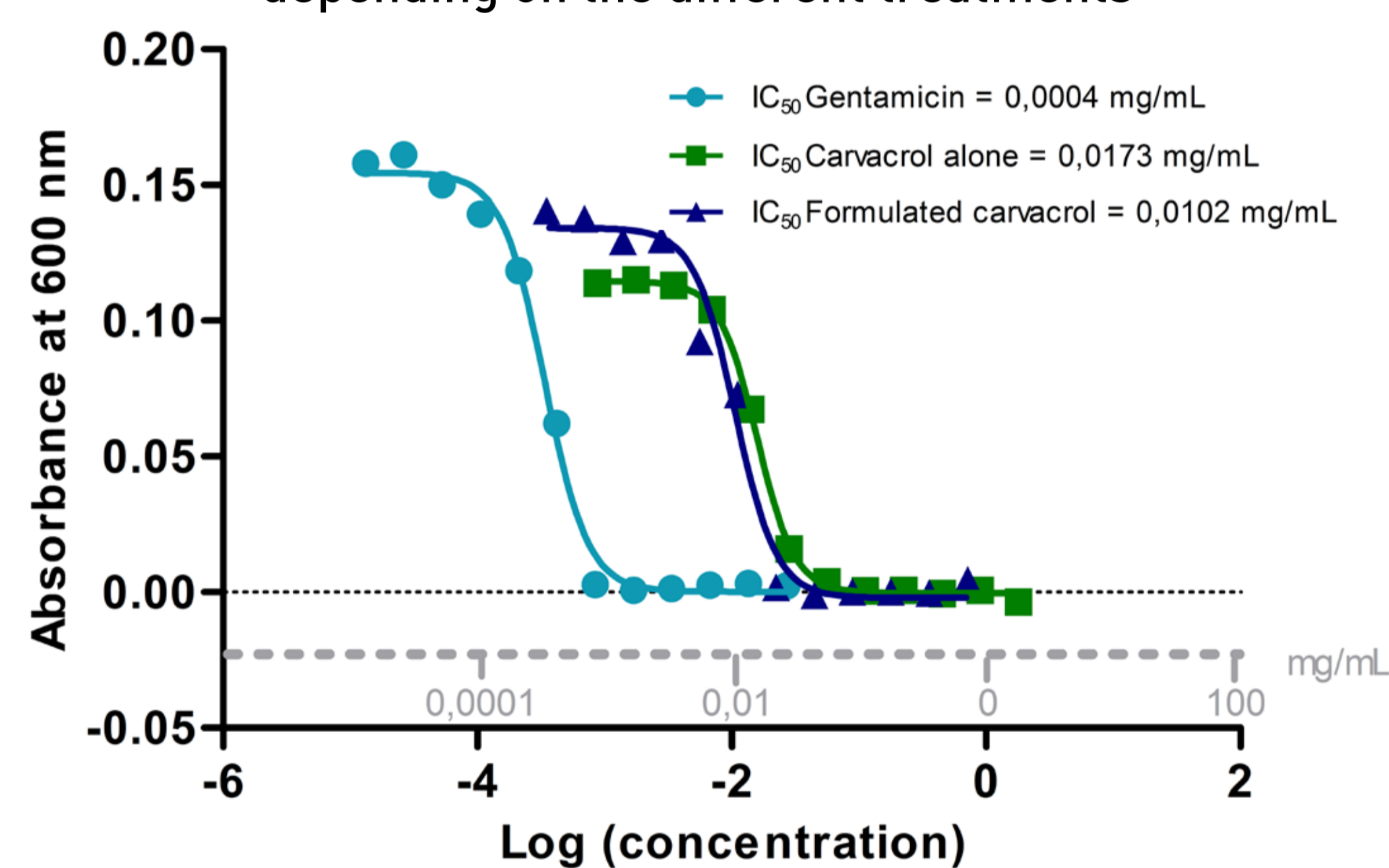
### MECHANISM OF ACTION ON MEMBRANE PERMEABILITY:

Treatment of *Campylobacter jejuni* cells with lethal doses of these 2 products during 3 hours and preparation of the samples for both Scanning (SEM) and Transmission (TEM) Electron Microscopy. Comparison with untreated *C. jejuni* cells.

## Results

### BROTH MICRODILUTION METHOD

Inhibition curves of the *Campylobacter jejuni* growth depending on the different treatments

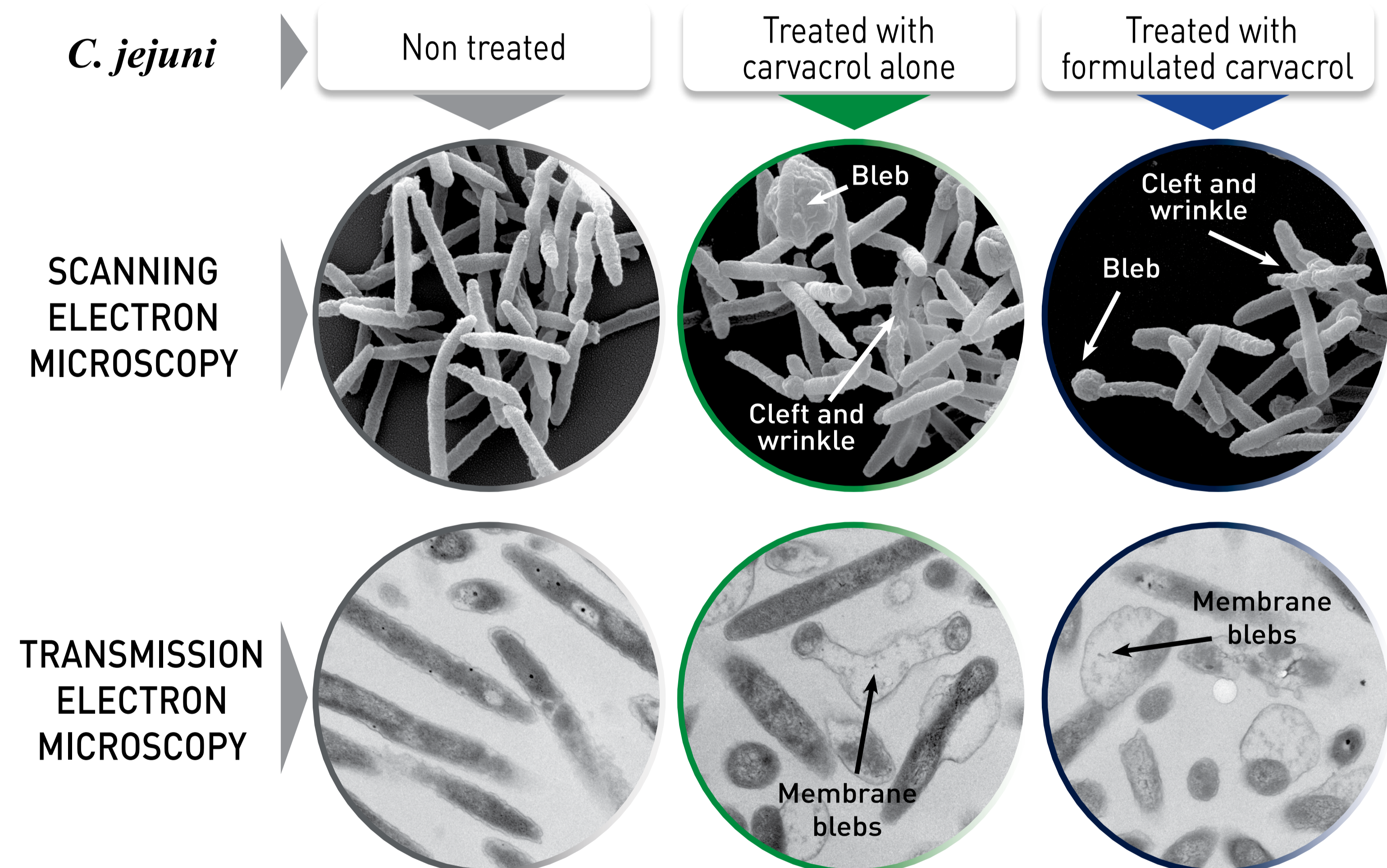


Products	MIC (mg/mL)	IC <sub>50</sub> (mg/mL)
Gentamicine	0,0008 ± 0,0004	0,0003 ± 0,0001 <sup>a</sup>
Carvacrol alone	0,0281 ± 0	0,0167 ± 0,006 <sup>b</sup>
Formulated carvacrol	0,0223 ± 0	0,0131 ± 0,0017 <sup>b</sup>

<sup>a,b</sup> P < 0.05

- ▶ Similar efficacy for carvacrol alone or formulated.
- ▶ Both products present an MIC of about 0,02 mg/mL and an IC<sub>50</sub> of about 0,01 mg/mL.

### *C. jejuni*



- **Untreated cells:** normal, with both spiraled and not spiraled shapes. Cells were smooth and without blebs.
- **Treated cells:** Similar results for liquid formulated carvacrol and pure carvacrol.
  - In SEM: wrinkles, clefts and blisters.
  - In TEM: membrane blebs caused by separation of the plasma membrane from outer membrane.
- ▶ **Membrane permeabilization.**

## Conclusion & Perspectives

- ▶ The liquid formulation does not change the efficacy of carvacrol against *Campylobacter jejuni in vitro*.
- ▶ Both pure carvacrol and formulated carvacrol induced a membrane permeability disruption.

### PERSPECTIVES

Confirm *in vivo* the efficacy of the liquid formulation linked on a solid carrier with two points :

- the ability of the product to reach the caeca
- the capacity of the product to kill *Campylobacter jejuni* cells in the caecal environment